

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

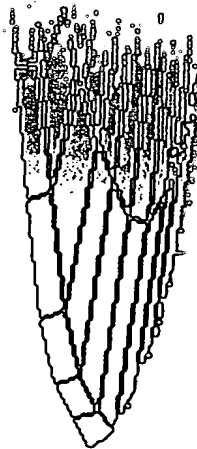
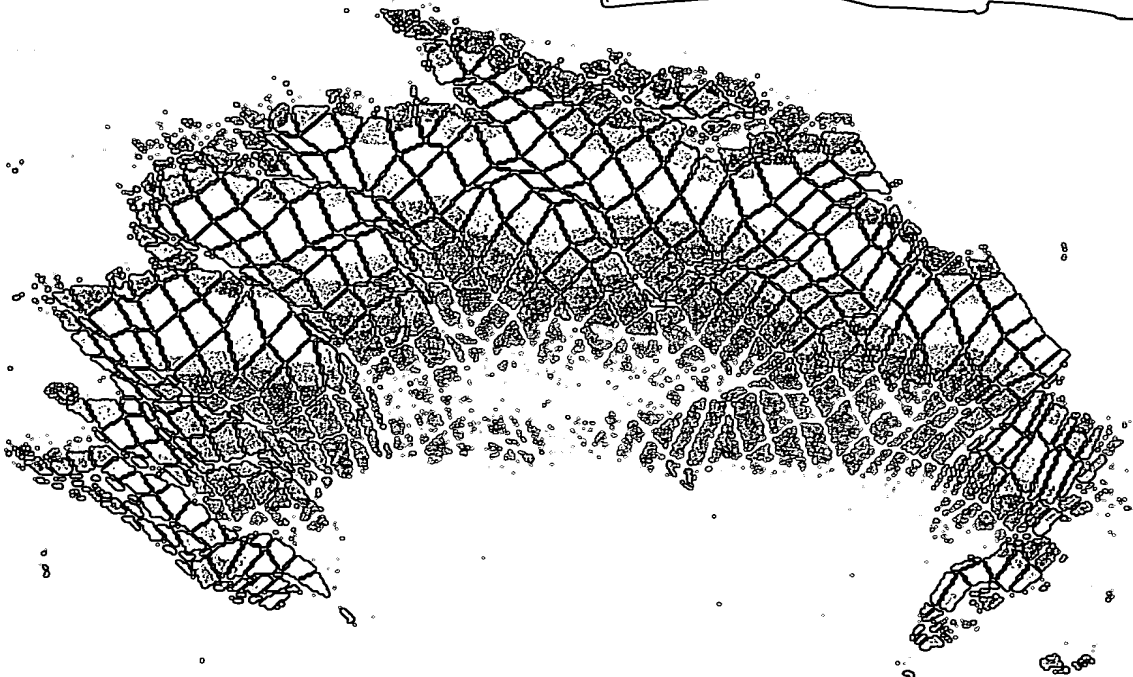
Defects in the images may include (but are not limited to):

- BLACK BORDERS
  - TEXT CUT OFF AT TOP, BOTTOM OR SIDES
  - FADED TEXT
  - ILLEGIBLE TEXT
- 
- SKEWED/SLANTED IMAGES
  - COLORED PHOTOS
  - BLACK OR VERY BLACK AND WHITE DARK PHOTOS
  - GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

As rescanning documents *will not* correct images,  
please do not report the images to the  
**Image Problem Mailbox.**

08XNCCNG \*\*\*\*\* FIRM 10032  
00000029091660AS 12/01/95 N-2895 14  
COLUMBIA UNIV  
HEALTH SCIENCES LIB  
701 N 168TH ST  
NEW YORK NY 10032-2704





**164**  
Risky science



**214**  
Pretty productive

## NEWS

- Physicists Create New State of Matter ☒ 152
- Plasma Physics: Go Back to Basics, Says NRC Panel 153
- More Than One Way to Fuse a Plasma 154
- Hughes Tosses Lifeline to 90 Eastern European Scientists 155
- Space Science: House Panel Targets Centers, Cassini 156
- Imanishi-Kari Case: Baltimore Defends Paper at Center of Misconduct Case 157
- Heavy Weather Ahead for Clinical Research 158
- New Faculty May Lose Family Tuition Help 158
- Marine Center Is Lightning Rod in Dispute Over Restoration 159
- Taking the Pulse of the Sun in Records of the Solar Wind 160
- How the T<sub>H</sub>2 Response Is Marshaled ☒ 161
- ## SPECIAL NEWS REPORT
- Epidemiology Faces Its Limits 164
- Sizing Up the Cancer Risks 165
- Press Coverage: Leaving Out the Big Picture 166

## PERSPECTIVES

- An Intimate Gathering of Bosons ☒ 18
- K. Burnett
- Methyl Chloroform and the Atmosphere ☒ 18
- A. R. Ravishankara and D. L. Albritton
- CD1: Presenting Unusual Antigens to Unusual T Lymphocytes ☒ 18
- A. Bendelac

## ARTICLE

- Atmospheric Trends and Lifetime of CH<sub>3</sub>CCl<sub>3</sub> and Global OH Concentrations ☒ 18
- R. G. Prinn, R. F. Weiss, B. R. Miller, J. Huang, F. N. Alyea, D. M. Cunnold, P. J. Fraser, D. E. Hartley, P. G. Simmonds

## RESEARCH ARTICLE

- Protein Folding Intermediates: Native-State Hydrogen Exchange 19
- Y. Bai, T. R. Sosnick, L. Mayne, S. W. Englander

## REPORTS

- Observation of Bose-Einstein Condensation in a Dilute Atomic Vapor ☒ 19
- M. H. Anderson, J. R. Ensher, M. R. Matthews, C. E. Wieman, E. A. Cornell

## THIS WEEK IN SCIENCE

- EDITORIAL 141
- The Politics of Science 143
- J. H. Gibbons
- LETTERS 145
- Succeeding Generations: C. T. Hill, J. Maddox, M. Heylin, E. Rubinstein, S. Mitton • Science and Political Reality: R. S. Walker • China's "Missing" Girls: T. O. Cheng, S. Tulljapourkar • New Light on Free Electron Lasers: A. Gover, W. van Amersfoort, W. B. Colson, 145

## DEPARTMENTS

- K. Mima, R. Warren • Applied Research in South Africa: G. F. R. Ellis • Correction: M. E. Gurney
- SCIENCESCOPE 151
- RANDOM SAMPLES 163
- BOOK REVIEWS 253
- Powering Apollo, reviewed by A. Roland • Vignettes • Reprints of Books • Books Received
- PRODUCTS & MATERIALS 255

## Board of Reviewing Editors

Frederick W. Alt  
Don L. Anderson  
Michael Ashburner  
Stephen J. Benkovic  
David E. Bloom  
Piet Borst  
Henry R. Bourne  
Michael S. Brown  
James J. Bull  
Kathryn Celame

C. Thomas Caskey  
Dennis W. Choi  
John M. Coffin  
F. Fleming Crim  
Paul J. Crutzen  
James E. Dahlberg  
Robert Desimone  
Paul T. Englund  
Richard G. Fairbanks  
Douglas T. Fearon

Harry A. Fozzard  
Klaus Friedrich  
Theodore H. Geballe  
Roger I. M. Glass  
Stephen P. Goff  
Peter N. Goodfellow  
Corey S. Goodman  
Ira Herskowitz  
Tomas Hökfelt  
Eric F. Johnson

Stephen M. Kosslyn  
Michael LaBarbera  
Nicole Le Douarin  
Charles S. Levings III  
Alexander Levitzki  
Harvey F. Lodish  
Richard Losick  
Reinhard Lührmann  
Diane Mathis  
Anthony R. Means

Shigetada Nakanishi  
Roger A. Nicoll  
Stuart L. Pimm  
Yeshayau Pocker  
Dennis A. Powers  
Ralph S. Quatrano  
V. Ramanathan  
Douglas C. Rees  
T. M. Rice  
David C. Rubie

Erkki Ruoslahti  
Gottfried Schatz  
Jozsef Schell  
Ronald H. Schwartz  
Terrence J. Sejnowski  
Ellen Solomon  
Thomas A. Steitz  
Michael P. Stryker  
Robert T. N. Tjian  
Emil R. Unanue

Geerat J. Vermeij  
Bert Vogelstein  
Arthur Weiss  
Zena Werb  
George M. Whitesides  
Owen N. Witte  
William A. Wulf

## COVER

color image of the velocity distribution in a cloud of sodium atoms that have formed a Bose-Einstein condensate. Color indicates the density of atoms having a velocity specified by the two horizontal axes. The density blue and white spire is an image of low-

energy atoms that have condensed into a single quantum state. The average speed of the atoms in the spire is about 0.5 millimeter per second. See page 198 and the related News story on page 152 and the Perspective on page 182. [Image: M. R. Matthews]



Strategy for a Convergent Synthesis of Linked Glycopeptides on a Solid Support 202  
Roberge, X. Beebe, S. J. Danishefsky

Motor-Enhanced Electron Transfer: Cytochrome c as a Redox-Inert Probe of Triplet Complexes 204  
Zhou, J. M. Nocek, M. L. DeVan, B. M. Man

Experimental Studies and Theoretical Calculations for the  $H + D_2 \rightarrow HD + D$  Reaction 207  
Kohnieder, K. Seekamp-Rahn, J. Borkowski, Wrede, K. H. Welge, F. J. Aoi, L. Bañares, D'Mello, V. J. Herrero, V. Sáez Rábanos, R. E. tt

Tree Records Covering the Last Glaciation 210  
Mowers and M. Bender

Isotopic Changes in the Partial Pressure of Carbon Dioxide in Coral Reef Water 214  
Meylanne, A. Suzuki, H. Saito

Stability of Diamond in Mantle Melt at High Pressure 216  
Mizuki, E. Ohtani, T. Kato

Related Reductions in Human Memory Due to Impaired Encoding 218  
Grady, A. R. McIntosh, B. Horwitz, J. M. Ng, L. G. Ungerleider, M. J. Mentis, P. Pietrini, Schapiro, J. V. Haxby

Activation of Human Leukocyte Tyrosine Kinases Through Protein-Coupled Receptors 221  
Knaus, S. Morris, H.-J. Dong, J. Chernoff, Bokoch

CD1 Binding and Presentation by CD1 223  
Castañón, S. Tangri, J. E. W. Miller, H. R. Imbe, M. R. Jackson, W. D. Huse, M. S. S. Berg, P. A. Peterson

CD1-Restricted T Cell Recognition of Microbial Lipoglycan Antigens 227  
P. A. Sieling, D. Chatterjee, S. A. Porcelli, T. I. Prigozy, R. J. Mazzaccaro, T. Soriano, B. R. Bloom, M. B. Brenner, M. Kronenberg, P. J. Brennan, R. L. Modlin

Targeted Disruption of Mouse EGF Receptor: Effect of Genetic Background on Mutant Phenotype 230  
D. W. Threadgill, A. A. Dlugosz, L. A. Hansen, T. Tennenbaum, U. Lichti, D. Yee, C. LaMantia, T. Mouton, K. Herrup, R. C. Harris, J. A. Barnard, S. H. Yuspa, R. J. Coffey, T. Magnuson

Strain-Dependent Epithelial Defects in Mice Lacking the EGF Receptor 234  
M. Sibilio and E. F. Wagner

Requirement of the Yeast *RTH1* 5' to 3' Exonuclease for the Stability of Simple Repetitive DNA 238  
R. E. Johnson, G. K. Kovvali, L. Prakash, S. Prakash

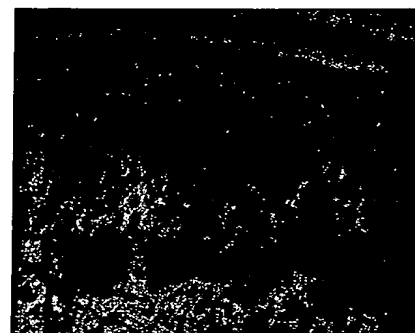
High Concentrations of Toxaphene in Fishes from a Subarctic Lake 240  
K. A. Kidd, D. W. Schindler, D. C. G. Muir, W. L. Lockhart, R. H. Hesselein

A Role in B Cell Activation for CD22 and the Protein Tyrosine Phosphatase SHP 242  
G. M. Doody, L. B. Justement, C. C. Delibrias, R. J. Matthews, J. Lin, M. L. Thomas, D. T. Fearon

Lack of Interferon  $\gamma$  Receptor  $\beta$  Chain and the Prevention of Interferon  $\gamma$  Signaling in  $T_H1$  Cells 245  
A. Pernis, S. Gupta, K. J. Gollob, E. Garfein, R. L. Coffman, C. Schindler, P. Rothman

## TECHNICAL COMMENTS

Seasonal Precipitation Timing and Ice Core Records 247  
C. D. Charles, D. Rind, J. Jouzel, R. D. Koster, R. G. Fairbanks



185, 223, & 227  
CD1 in skin

## Board of Directors

I. Ayala  
President  
n  
hwel  
it  
anco  
it-elect

ester Jr.  
win  
lovacek

Anna C. Roosevelt  
Alan Schriesheim  
Jean E. Taylor  
Chang-Lin Tien  
Nancy S. Wexler

William T. Golden  
Treasurer  
Richard S. Nicholson  
Executive Officer

■ SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1333 H Street, NW, Washington, DC 20005. Second-class postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 1995 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$97 (\$50 allocated to subscription). Domestic institutional subscription (51 issues): \$228. Foreign postage extra: Mexico, Caribbean (surface mail) \$53; other countries (air assist delivery) \$93. First class, airmail, student and emeritus rates on request. Canadian rates with GST available upon request. GST #1254 88122. Printed in the U.S.A.

Change of address: allow 6 weeks, giving old and new addresses and 11-digit account number. Postmaster: Send change of address to Science, P.O. Box 2033, Marion, OH 43305-2033. Single copy sales: \$7.00 per issue prepaid includes surface postage; bulk rates on request. Authorization to photocopy material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that \$3.00 per article is paid directly to CCC, 27 Congress Street, Salem, MA 01970. The identification code for Science is 0036-8075/95 \$3.00. Science is indexed in the Reader's Guide to Periodical Literature and in several specialized indexes.

# A Strategy for a Convergent Synthesis of N-Linked Glycopeptides on a Solid Support

Jacques Y. Roberge, Xenia Beebe, Samuel J. Danishefsky

Oligosaccharides and glycopeptides are of considerable importance in molecular biology and pharmacology. However, their synthesis is complicated by the large number of different linking sites between each saccharide unit, the need for stereochemical control, the chemical sensitivity of the glycopeptide bonds, and the need to harmonize diverse protecting groups. Here, an efficient solid-phase synthesis of three N-linked glycopeptides based on glycal assembly is presented. The peptide domain can be extended while the ensemble remains bound to the polymer. The glycopeptides synthesized here are among the largest N-linked glycopeptides ever accessed by either solution- or solid-phase synthesis.

The surge of interest in glycoproteins (1-3) arises from heightened awareness of their importance in diverse biochemical processes, including cell growth regulation, binding of pathogens to cells (4), intercellular communication, and metastasis (5). Glycoproteins serve as cell differentiation markers and assist in protein folding and transport, possibly by providing protection against proteolysis (6). Improved isolation techniques and structural elucidation methods (7) have revealed high levels of microheterogeneity in naturally produced glycoproteins (8). Single eukaryotic cell lines often produce many glycoforms of any given protein sequence. For instance, erythropoietin, a clinically useful red blood cell stimulant against anemia, is glycosylated by more than 13 known types of oligosaccharide chains when expressed in Chinese hamster ovary cells (9). The efficacy of erythropoietin is heavily dependent on the type and extent of glycosylation (10).

Elucidation of the biological relevance of particular glycoprotein oligosaccharide chains benefits from isolation of pure entities. Glycoprotein heterogeneity renders this process particularly labor intensive. Some relief may be in sight in that particular cell lines can be selected to produce more homogeneous glycoproteins for structure-activity relation studies (11). However, the problem of isolation from natural sources remains daunting.

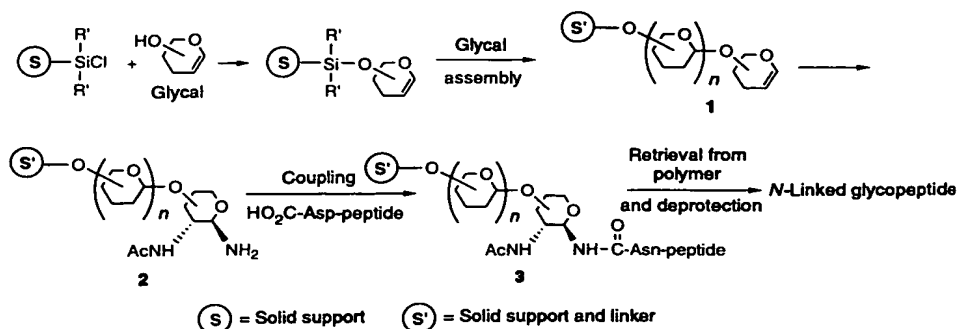
Fortunately, receptors normally recognize only a small fraction of a given macromolecular glycoconjugate. Consequently, synthesis of smaller but well-defined putative glycopeptide ligands could emerge as competitive with isolation as a source of

critical structural information (9). Important progress in glycopeptide synthesis pioneered by Kunz and others allowed synthetic access to homogenous target systems both in solution and in the solid phase (2, 12-15). Cohen-Anisfeld and Lansbury have recently reported a highly convergent solution-based coupling of selected already-available saccharides with peptides (13). In our method, the terminal glycal on the solid phase is linked with a peptide domain to generate an asparagine-linked N-acetylglucosamine construct; the whole ensemble is then retrieved and deblocked. This procedure allows one to fashion the carbohydrate domain of choice and also benefits from the

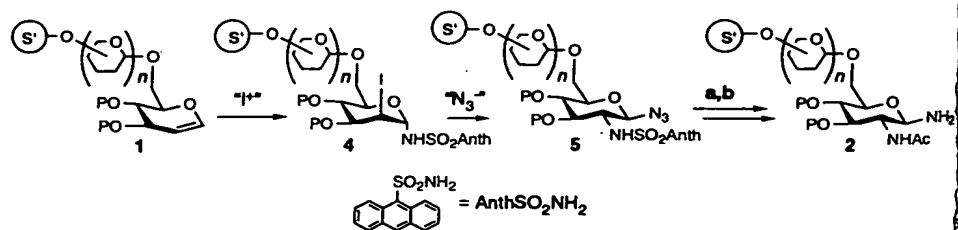
advantages associated with solid-phase synthesis in the critical carbohydrate-peptide coupling step.

The most successful approach to solid phase N-linked glycopeptide synthesis in current practice involves construction of peptide segment bearing an amine at the terminal residue on a solid support. The amine is then deprotected and coupled with either an appropriate oligosaccharide or small glycopeptide. Cleavage from the solid support and deprotection yields the desired glycopeptide (12).

The method we propose is illustrated in Scheme 1. An oligosaccharide terminating in a glycal is constructed on the solid support (see structure 1), which can be an extended linear structure or can contain branching as desired (16, 17). Through chemistry described below (Scheme 2), 1 is converted to the solid phase-bound 2, bearing a terminal 2-N-acetyl-1-β-aminoglucosamine residue (GlcNAc). A peptide is readily assembled through standard solution-phase peptide synthesis methodology or by a solid-phase assembly-retrieval sequence. Coupling of 2 with a suitable aspartic acid-containing peptide affords solid phase-bound glycopeptide 3. Retrieval and full deprotection affords the desired N-linked glycopeptide. In addition, deprotection of the carboxy terminus and addition of a peptide with a free amino terminus allows for elongation of the peptide chain while the glycopep-



Scheme 1. N-Linked glycopeptide synthesis through the use of polymer-bound glycals.



Scheme 2. Preparation of polymer-bound 2-N-acetyl-1-β-amino glucosylamine by the azasulfonamidation reaction. P = saccharide protecting group; "I<sup>+</sup>" = iodonium bis(collidine) perchlorate; "N<sub>3</sub><sup>-</sup>" = tetrabutylammonium azide (Bu<sub>4</sub>NN<sub>3</sub>); Anth = anthracene; [a] acylation with acetic anhydride (Ac<sub>2</sub>O) and 4-N,N-dimethylaminopyridine (DMAP); [b] reduction with 1,3-propanedithiol and N,N-diisopropyl-N'-ethylamine (i-Pr<sub>2</sub>NEt).

J. Y. Roberge and X. Beebe, Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, Box 106, New York, NY 10021, USA.

S. J. Danishefsky, Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, Box 106, New York, NY 10021, and Department of Chemistry, Columbia University, New York, NY 10027, USA.

de 3 is still bound to the solid support.

A simple sequence was devised to convert 1 to 2 (Scheme 2). The use of the anthracenesulfonamide (18) in the azasulfonamidation sequence (19) was crucial for the addition step (4), the azide-induced rearrangement (5), and the presentation of the solid phase-bound GlcNAc residue bearing a 1 $\beta$ -amino function (2). Two examples of this design for glycopeptide syn-

thesis are illustrated in Scheme 3, showing the relative simplicity of protecting-group requirements and the high order of convergence of the approach. They result, after deprotection, in 22 or 23.

The principal advantage in using the anthracenesulfonamide is that the nitrogen-sulfur linkage can be cleaved by a variety of mild methods (18). For instance, we developed the use of thiophenol or 1,3-

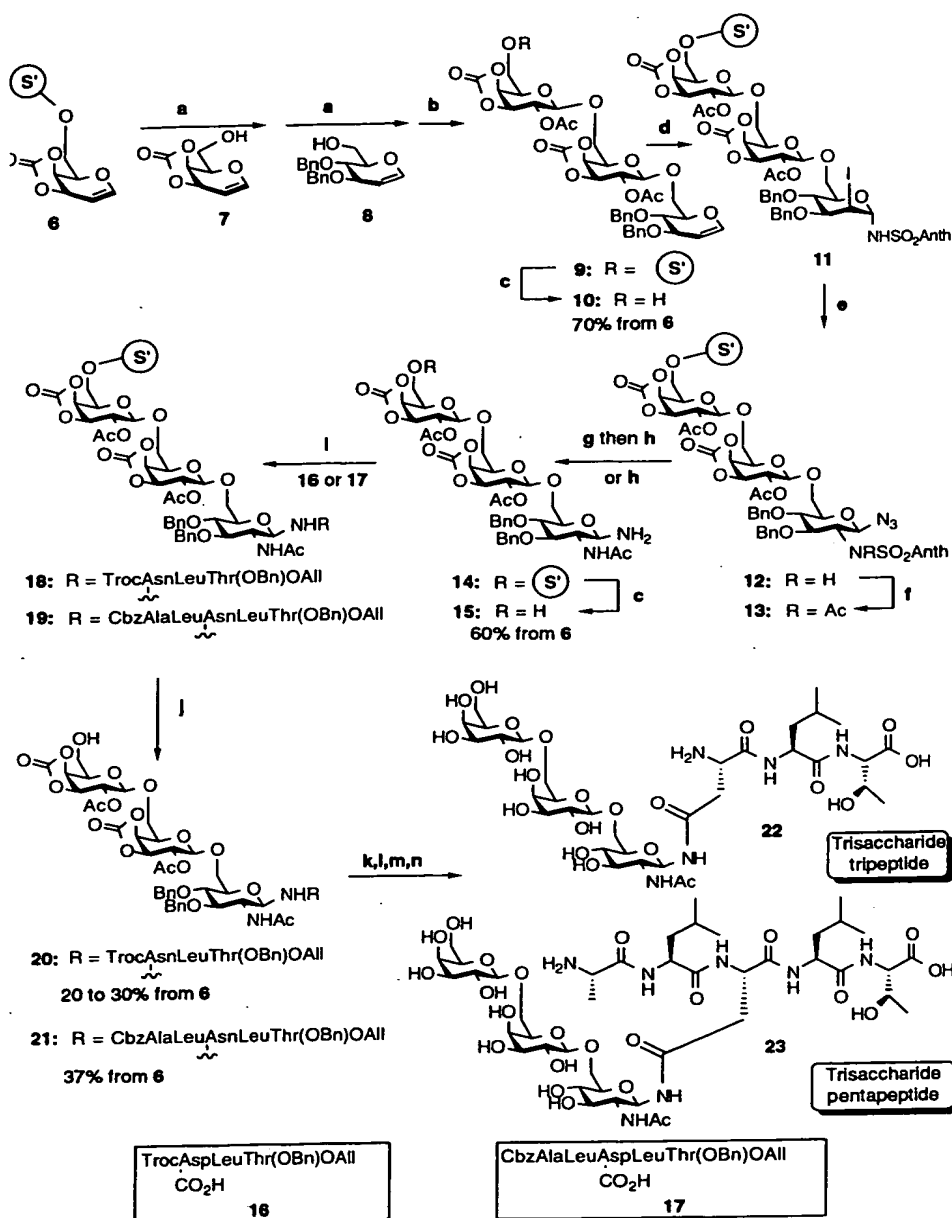
propanedithiol and Hunig's base for the removal of the anthracenesulfonyl group. These protocols are compatible with the solid-supported synthesis. Also, anthracenesulfonamide itself is more soluble than benzenesulfonamide in tetrahydrofuran (THF), which is a good swelling solvent for the polymer-supported work. Thus, the use of the anthracene-based agent results in a more efficient and complete iodosulfonamidation reaction.

In solution-phase coupling of carbohydrates and peptides, the process of separating the unreacted components and by-products is not a trivial matter. Purification is greatly simplified by conducting the coupling reaction on the solid support. Most of the excess peptide is recovered from the washing by chromatography. Small amounts are lost when the activated aspartic residue cyclizes to an aspartimide. In practice, protected trisaccharide pentapeptide 21 was obtained in 37% overall yield on the basis of the initial loading of galactal carbonate. The average yield for each of the 10 steps of the sequence to 21 was 91%. Chromatography on a short column of reverse-phase silica (octadecylsilane) was sufficient to obtain this compound in pure form. This purification capability arises from the previously described "self-policing" feature of the solid-phase glycal assembly method, which avoids deletions through destruction of uncoupled donors before the next coupling cycle (17).

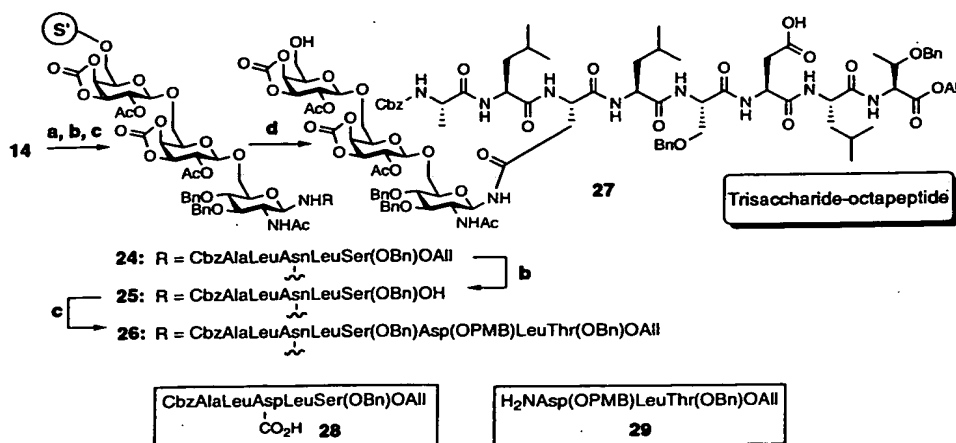
The glycopeptides retrieved from the support were deprotected as shown and the fully deblocked glycopeptides 22 and 23 were obtained in 61% and 48% overall yields from 20 and 21, respectively. Structural characterization of the glycopeptides by nuclear magnetic resonance spectroscopy showed the  $\beta$  configuration of all the anomeric linkages. The structures were further corroborated by mass spectroscopy.

The presence of orthogonal protecting groups on the carboxyl and amino termini of the peptide provides the opportunity to extend the peptide chain while the ensemble is bound to the solid support. Alternatively, after removal from the support, the freed peptide terminus may provide a functionality for linking to a carrier molecule to generate other glycoconjugates (20). Scheme 4 shows how the peptide portion of the glycopeptide was extended while still bound to the polymer support. Solid phase-bound trisaccharide pentapeptide 24 was assembled and the carboxyl terminus deprotected to give the acid, 25. Polymer-bound 25 was then coupled to tripeptide 29 with a free amino terminus to give glycopeptide 26. Retrieval from the solid support afforded trisaccharide octapeptide 27 in an 18% overall yield from polymer-bound galactal carbonate.

Regarding the future of glycopeptide



**Scheme 3.** Solid-phase synthesis of *N*-linked glycopeptides. All = allyl; Troc = 2,2,2-trichloroethoxy-carbonyl; Cbz = benzyloxycarbonyl; Bn = benzyl. [a] Dimethyldioxirane, THF (23), 7 or 8, zinc chloride, and THF; [b] Ac<sub>2</sub>O, collidine, DMAP, and THF; [c] tetrabutylammoniumfluoride, acetic acid, and THF at 0°C; [d] iodonium bis(collidine) perchlorate (24) and AnthSO<sub>2</sub>NH<sub>2</sub> (9 → 11); [e] Bu<sub>4</sub>NN<sub>3</sub> and THF (11 → 2); [f] Ac<sub>2</sub>O, DMAP, and THF; [g] thiophenol, *i*-Pr<sub>2</sub>NEt, and THF; [h] 1,3-propanedithiol, *i*-Pr<sub>2</sub>NEt (14), and THF (13 → 14); [i] 1-isobutoxy-2-isobutoxycarbonyl-1,2-dihydroquinoline (IIDQ), CH<sub>2</sub>Cl<sub>2</sub>, and 16 or 7 (14 + 16 → 18 and 14 + 17 → 19); [j] HF-pyridine, anisole (25), and THF (18 → 20 and 19 → 21); [k] d(PPh<sub>3</sub>)<sub>4</sub>, dimethylbarbituric acid, and THF (26); [l] Zn, acetic acid, and methanol (26); [m] H<sub>2</sub>, Pd(OAc)<sub>2</sub>, and methanol (27); [n] KCN and methanol. Yields 22 (61% from 20) or 23 (48% from 21) (28).



**Scheme 4.** Extension of the peptide portion of the glycopeptide on the solid support. PMB = *p*-methoxybenzyl. [a] **28**, IIDQ, and CH<sub>2</sub>Cl<sub>2</sub> (**14** → **24**); [b] Pd(PPh<sub>3</sub>)<sub>4</sub>, dimethylbarbituric acid, and THF; [c] **29**, IIDQ, and CH<sub>2</sub>Cl<sub>2</sub>; [d] HF-pyridine, anisole, and CH<sub>2</sub>Cl<sub>2</sub> (**26** → **27**).

synthesis, one should also consider the method of Wong, which involves enzymatically mediated elaboration of a solid phase-bound glycosylated peptide construct using glycosyl transferases to append unprotected nucleoside phosphate-activated monosaccharides in the elongation phase (21). By this method, retrieval from the solid support is mediated by protease action. The relief from protecting groups in enzymatically mediated chemistry can be a substantial advantage. Both Wong's method and our method allow the use of unnatural amino acids and non-amino acids. The method described herein is, in principle, totally general in that it does not require the existence of the transferases and the availability of nucleoside-activated hexoses. It can also accommodate the inclusion of unnatural (artificial) sugars in the scheme. Such building blocks are available from the Lewis acid-catalyzed diene-aldehyde cyclocondensation reaction (22). All workable approaches, whether purely chemical or chemoenzymatic, are complementary for reaching the common goal of carefully designed, fully synthetic glycopeptides.

## REFERENCES AND NOTES

- M. J. McPherson, P. Quirke, G. R. Taylor, Eds., *PCR: A Practical Approach* (IRL Press, Oxford, UK, 1991); G. M. Blackburn and M. J. Gait, Eds., *Nucleic Acids in Chemistry and Biology* (IRL Press, Oxford, UK, 1990); A. M. Bray, A. G. Jhingran, R. M. Valerio, N. J. Maaji, *J. Org. Chem.* **59**, 2197 (1994); G. Jung and A. G. Beck-Sickinger, *Angew. Chem. Int. Ed. Engl.* **31**, 367 (1992); M. A. Gallop, R. W. Barrett, W. J. Dower, S. P. A. Fodor, E. M. Gordon, *J. Med. Chem.* **37**, 1233 (1994); H. P. Nestler, P. A. Bartlett, W. C. Still, *J. Org. Chem.* **59**, 4723 (1994).
- M. Meldal, *Curr. Opin. Struct. Biol.* **4**, 710 (1994).
- T. Feizi and D. Bundle, *ibid.*, p. 673.
- O. P. Bahl, in *Glycoconjugates: Composition, Structure, and Function*, H. J. Allen and E. C. Kisailus, Eds. (Dekker, New York, 1992), p. 1-12.
- A. Kobata, *Acc. Chem. Res.* **26**, 319 (1993).
- G. Opdenakker, P. M. Rudd, C. P. Ponting, R. A.

Dwek, *FASEB J.* **7**, 1330 (1993).

- A. Dell and K.-H. Khoo, *Curr. Opin. Struct. Biol.* **3**, 687 (1993).
- R. A. Dwek, C. J. Edge, D. J. Harvey, M. R. Wormald, R. B. Parekh, *Annu. Rev. Biochem.* **62**, 65 (1993).
- Y. C. Lee and R. T. Lee, Eds., *Neoglycoconjugates: Preparation and Applications* (Academic Press, London, 1994).
- E. Watson, A. Bhide, H. van Halbeek, *Glycobiology* **4**, 227 (1994).
- M. A. Lehman and Z. Yucheng, U.S. Patent 5 272 070 A (1993).
- M. Meldal, in (9), p. 145; S. J. Danishefsky and J. Y. Roberge, in *Glycopeptides and Related Compounds: Chemical Synthesis, Analysis and Applications*, D. G. Large and C. D. Warren, Eds. (Dekker, New York, in press).

- S. T. Cohen-Anisfeld and P. T. Lansbury Jr., *J. Am. Chem. Soc.* **115**, 10531 (1993).
- S. T. Anisfeld and P. T. Lansbury Jr., *J. Org. Chem.* **55**, 5560 (1990).
- D. Vetter, D. Tumelty, S. K. Singh, M. A. Gallop, *Angew. Chem. Int. Ed. Engl.* **34**, 60 (1995).
- J. T. Randolph, K. F. McClure, S. J. Danishefsky, *J. Am. Chem. Soc.*, in press; J. T. Randolph and S. J. Danishefsky, *Angew. Chem. Int. Ed. Engl.* **33**, 1470 (1994); S. J. Danishefsky, J. T. Randolph, J. Y. Roberge, K. F. McClure, R. B. Ruggeri, in *The Schering Lecture Series* (Schering, Germany, 1995); *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.* **35**, 977 (1994).
- S. J. Danishefsky, K. F. McClure, J. T. Randolph, R. B. Ruggeri, *Science* **260**, 1307 (1993).
- A. J. Robinson and P. B. Wyatt, *Tetrahedron* **49**, 11329 (1993).
- F. E. McDonald and S. J. Danishefsky, *J. Org. Chem.* **57**, 7001 (1992).
- C. Unverzagt and H. Kunz, *Bioorg. Med. Chem.* **3**, 197 (1993).
- M. Schuster, P. Wang, J. C. Paulson, C.-H. Wong, *J. Am. Chem. Soc.* **116**, 1135 (1994).
- S. J. Danishefsky, *Chemtracts Org. Chem.* **2**, 273 (1989); D. B. Berkowitz, S. J. Danishefsky, G. K. Schulte, *J. Am. Chem. Soc.* **114**, 4518 (1992).
- The polymer-bound disaccharide **18** was prepared from polymer-bound galactal carbonate and 3,4-galactal carbonate according to a procedure described in (17).
- R. U. Lemieux and S. Lavine, *Can. J. Chem.* **42**, 1473 (1964).
- S. Matsuura, C.-H. Niu, J. N. Cohen, *Chem. Commun.* **1976**, 451 (1976).
- H. Kunz and J. März, *Synlett* **1992**, 591 (1992).
- D. A. Jones Jr., *Tetrahedron Lett.* **33**, 2853 (1977).
- H. Paulsen, M. Schultze, D. Klamann, B. Waller, M. Paal, *Liebigs. Ann. Chem.* **1985**, 2028 (1985).
- This research was supported by NIH grant AI 16943. We also acknowledge a National Science and Engineering Research Council (Canada) postdoctoral fellowship to J.Y.R. and a NIH postdoctoral training grant T32 CA62948 to X.B.

9 February 1995; accepted 19 May 1995

## Inhibitor-Enhanced Electron Transfer: Copper Cytochrome c as a Redox-Inert Probe of Ternary Complexes

Jian S. Zhou, Judith M. Nocek, Michael L. DeVan, Brian M. Hoffman\*

Copper-substituted cytochrome c (CuCc) has been used as a structurally faithful, redox-inert inhibitor to probe the mechanism of electron transfer (ET) between Cc molecules and cytochrome c peroxidase (CcP). This inhibitor enhances photoinduced ET quenching of the triplet excited state of a zinc-substituted protein (ZnCcP or ZnCc) by its iron(III) partner (Fe<sup>3+</sup>Cc or Fe<sup>3+</sup>CcP). These results show that CcP and Cc form a ternary complex in which one Cc molecule binds tightly at a surface domain of CcP having low ET reactivity, whereas the second Cc molecule binds weakly to the 1:1 complex at a second domain with markedly greater (~10<sup>3</sup>) reactivity. These results also rule out the possibility that Cc bound at the second domain cooperatively enhances ET to Cc at the first domain. The multiphasic kinetics observed for the photoproduced ET intermediate do not reflect electron self-exchange between two Cc molecules within the ternary complex.

Respiration and metabolism depend on the sequential transfer of electrons from one protein to another (1), and this ET in turn

Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA.

\*To whom correspondence should be addressed.

depends on the recognition and docking, as well as the reaction, of the ET partners (2). Issues of binding specificity and of reactivity in protein-protein reactions are analogous to those in enzyme-substrate reactions, but studies of ET complexes have lacked paral-